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Differential Prefrontal White Matter Development in Chimpanzees and Humans

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Summary

A comparison of developmental patterns of white matter (WM) within the prefrontal region between humans and non-human primates is key to understanding human brain evolution. WM mediates complex cognitive processes, and has reciprocal connections with posterior processing regions [1-2]. Although the developmental pattern of prefrontal WM in macaques differs markedly from that in humans [3], this has not been explored in our closest evolutionary relative, the chimpanzee. The present longitudinal study of magnetic resonance imaging (MRI) scans demonstrated that the prefrontal WM volume in chimpanzees was immature and had not reached the adult value during pre-puberty, as observed in humans but not in macaques. However, the rate of prefrontal WM volume increase during infancy was slower in chimpanzees than in humans. These results suggested that a less mature and more protracted elaboration of neuronal connections in the prefrontal portion of the developing brain existed in the last common ancestor of chimpanzees and humans, and that this served to enhance the impact of postnatal experiences on neuronal connectivity. Furthermore, the rapid development of the human prefrontal WM during infancy may help the development of complex social interactions, as well as the acquisition of experience-dependent knowledge and skills to shape neuronal connectivity.

Results

We obtained longitudinal T1-weighted magnetic resonance imaging (MRI) scans at scheduled intervals from three growing chimpanzees (*Pan troglodytes*) between the ages of 6 months and 6 years (the middle of early infancy and the second half of the juvenile stage, respectively; 6 years is still considered the pre-pubertal stage in chimpanzees) (**Supplemental Figure1** (**Figure S1**)). The prefrontal and non-prefrontal portions of the cerebrum were divided by the coronal slice anterior to the corpus callosum, as a proxy for the prefrontal volume on MRI data, in accordance with previous neuroimaging studies [4-7]. The rationale for this definition is explained in the **Supplemental Discussion**. Gray and white matter (GM, WM) volumes in the prefrontal and non-prefrontal portions were calculated based on a segmentation map generated with FSL software [8] (**Supplemental Table**). The results were compared with those of humans (*Homo sapiens*) between the ages of 1 month and 10.5 years, corresponding to the near onset of early infancy and the second half of the juvenile stage, respectively; 10.5 years is still considered the pre-pubertal stage in humans based on sexual maturation (**Figure S1**) [9] [Matsui et al., the 20th annual Rotman Research Institute Conference- "Frontal Lobes", Abstract, 2010]. The results were also compared with those of rhesus macaques (*Macaca mulatta*) between the ages of 10 months and 5.3 years, corresponding to the middle of late infancy and the near onset of adult stages, respectively (**Figure S1**) [3]. A more detailed account of the materials and methods is available in **Supplemental Experimental Procedures** and **Supplemental Discussion**.

Total and prefrontal tissue volumes

The results of tissue segmentation revealed noteworthy developmental changes in chimpanzees through the study period (**Figure 1**). The total (GM plus WM) volumes of each of the prefrontal and non-prefrontal portions increased non-linearly ($F = 7.11$; quadratic effect, $P < 0.01$; $F = 27.76$; cubic effect, $P < 0.0001$) (**Figure 2A**). The non-prefrontal GM volume followed a non-linear developmental trajectory with the maximum value at about 3 years (the end of late infancy) and decreased gradually thereafter ($F = 8.73$; cubic effect, $P < 0.001$), whereas the prefrontal GM volume continued to increase gradually with age ($F = 4.31$; quadratic effect, $P < 0.05$) (**Figure 2C**). The WM volumes in the prefrontal and non-prefrontal portions increased non-linearly ($F = 11.15$; cubic effect, $P < 0.001$; $F = 34.05$; cubic effect, $P < 0.0001$) (**Figure 2B**).

Chimpanzees and humans differ from macaques in the delayed development of WM volume, especially of the prefrontal portion. The WM volumes of the prefrontal and non-prefrontal portions of the chimpanzee were 60.0% and 71.1%, respectively, of the adult volume (**Supplemental Experimental Procedures**) at the second half of the juvenile stage (6 years) (**Figure 3A**). In humans, the WM volumes of the prefrontal and non-prefrontal portions were 85.1% and 79.1% of the adult volume at almost the same developmental stage (10.5 years), respectively (**Figure 3B**).

By marked contrast, the corresponding WM volumes of macaques reached the adult volume at 1.9 and 2.9 years, respectively (**Figure 3C**). These ages are pre-pubertal stages and correspond to the first half of the juvenile stage and near the end of the juvenile stage, respectively. The prefrontal WM volume reached the developmental peak at 2.9 years, and then decreased from puberty onwards (**Figure S3B**).

However, the chimpanzee WM volumes of the prefrontal and non-prefrontal portions in the second half of the juvenile stage were lower than those of humans (**Figure 3A-B**). This difference can be attributed to differences in the rates of WM volume increases during infancy between the two species (**Supplemental Discussion**). The WM volumes of the prefrontal and non-prefrontal portions in chimpanzees increased 173.4% and 62.3%, respectively, across the developmental stage from the middle of early infancy to the end of late infancy (6 months to 3 years) (**Figure 2B**). By contrast, the WM volumes of the prefrontal and non-prefrontal portions in humans increased 185.2 % and 79.7 %, respectively, during nearly the same developmental stage (1 year to 6 years) (**Figure S2B**). On the other hand, the corresponding WM volumes in macaques increased by only 24.6% and 21.8% from the middle of late infancy to the end of the juvenile stage (10 months to 3.2 years).

Proportional growth of WM volume

In chimpanzees, the developmental trajectories of the total volume in the prefrontal portion differed from those of the non-prefrontal portion, not observed in either humans or macaques (**Figure 2A**, **Figure S2A**, and **Figure S3A**). Differences in the prefrontal and non-prefrontal developmental patterns of WM volume appear to greatly influence the differences in the total volume of the adult prefrontal portion. Thus, to elucidate species-specific variations in chimpanzees, humans, and macaques, we evaluated the proportional growth of the WM volume relative to the total volume of the developing cerebrum and compared the result to the adult value. The proportional growth of WM volume was calculated by dividing WM volume expressed as a percentage of the total volume of GM plus WM in the prefrontal and non-prefrontal portions by the adult percentage.

The proportional growth of WM volume of the prefrontal and non-prefrontal portions in chimpanzees significantly increased, following a cubic and quadratic curve, respectively ($F = 36.54$; cubic effect, $P < 0.0001$; $F = 26.18$; quadratic effect, $P < 0.0001$) (**Figure 4A**). The proportional increase in WM volume in the prefrontal portion was significantly smaller than that in the non-prefrontal portion (ANCOVA; $F = 74.60$; $P < 0.0001$).

As observed in humans, the chimpanzee cerebral WM volume at an early developmental stage was immature compared to the adult volume, especially in the prefrontal portion. At the middle of early infancy (6 months), the proportional WM volume in the prefrontal and non-prefrontal portions was 25.5% and 50.9%, respectively (**Figure 4A**). The corresponding WM volumes in humans at approximately the same developmental stage (1 year) were 33.0% and 53.4%, respectively (**Figure 4B**).

Chimpanzees also share with humans a common protracted developmental trajectory of WM volume, especially in the prefrontal portion during infancy and the juvenile stage. In the second half of the juvenile stage (6 years), the proportional WM volume in the prefrontal and non-prefrontal portions was 55.7% and 73.6%, respectively, and had not yet attained the adult value (**Figure 4A**). The corresponding values in humans at approximately the same developmental stage (10.5 years) were 83.9% and 84.3% of the adult value, respectively, and had also not yet attained the adult value (**Figure 4B**). In contrast, the proportional WM volume in the prefrontal and non-prefrontal portions of macaques had already reached a plateau at an early developmental stage: the prefrontal and non-prefrontal proportional values were 91.7% and 90.8% of the adult value, respectively, at the end of late infancy (1.3 years), and 96.5% and 97.1%, respectively, at the end of the juvenile stage (3.2 years) (**Figure 4C**).

Although chimpanzees and humans shared a markedly immature prefrontal WM volume at the middle of early infancy, the prefrontal WM volume in chimpanzee infants developed along a slower trajectory compared with human infants. In chimpanzees, the proportional WM volume in the prefrontal portion reached 31.8% of the adult value at the end of early infancy (1 year), 49.7% at the end of late infancy (3 years), and 55.7% in the second half of the juvenile stage (6 years) (**Figure 4A**). The proportional WM volume of the non-prefrontal portion was 54.0%, 64.2%, and 73.6% at each respective developmental stage (**Figure 4A**). By contrast, in humans, the proportional WM volume in the prefrontal portion attained 48.9% of the adult value at the end of early infancy (2 years), 76.2% at the end of late infancy (6 years), and 83.9% in the second half of the juvenile stage (10.5 years) (**Figure 4B**). The corresponding values of the non-prefrontal portion were 63.6%, 77.2%, and 84.3% at each respective developmental stage (**Figure 4B**).

Discussion

The human prefrontal cortex mediates evolutionarily advanced functions such as working memory, temporal integration, motivation, decision-making, self-awareness, creativity, language, and social interaction [1-2]. The executive role played by the prefrontal region critically depends on its reciprocal connections to the diencephalon, mesencephalon, and limbic systems, as well as numerous brain regions that mediate higher sensory functions [1, 6]. Therefore, focusing on the augmentation of WM volume in the prefrontal region, which is attributable to the myelination of axons that connect the posterior processing regions [1], is key to understanding human brain evolution.

Whether or not the human prefrontal region or any of its subdivisions is proportionally enlarged remains controversial [6, 10-18]. In particular, comparative studies

have not reached a consensus as to whether the prefrontal WM enlargement is an evolutionary specialization of humans [6, 18]. Unfortunately, few studies have compared the developmental patterns of the prefrontal region in humans and non-human primates. Moreover, brain developmental patterns have not thus far been explored in the great apes. An understanding of the developmental processes in the prefrontal region is expected to provide powerful insights into the evolution of adult brain morphologies and function.

We performed the first longitudinal analysis of brain developmental trajectory in the closest primate relative of humans, the chimpanzee. Despite the relatively small sample size in the current chimpanzee study, our results successfully illustrate similarities and differences in brain volume trajectories among chimpanzees, humans, and rhesus macaques. The prefrontal WM maturation in chimpanzees, as in humans, showed a less mature and more protracted course compared with the maturation of the non-prefrontal portion throughout pre-puberty. However, the rate of increase of prefrontal WM volume during infancy was slower in chimpanzees that in humans.

Recent imaging studies of human brain development emphasized the more protracted course of development in the frontal region, based on the timing in which the cortex and fiber trajectories matured. A recent MRI study investigated the development of the cortical GM density and reported that maturation of the frontal region, a high-order association area, progresses in a back-to-front direction [19]. The progression begins in the low-order sensorimotor regions and spreads anteriorly over the superior and inferior frontal gyri, with the prefrontal region developing last. We demonstrated that, in a similar fashion, regional differences of WM maturation between the prefrontal and non-prefrontal portions in chimpanzees appeared to be involved in the differential maturation between the prefrontal executive function and the sensory and motor systems after birth.

Previous developmental diffusion tensor imaging (DTI) studies indicated that certain frontal connections in humans, particularly fronto-temporal connections such as the uncinate fasciculus and the cingulum, tend to mature more slowly than the projection connections and the other association connections [20-21]. The patterns of connection elaboration of chimpanzees in this study appeared to reflect the same phenomena. We infer that the protracted connection maturation of the prefrontal portions in human and chimpanzees is attributable to the elaborations of reciprocal cortico-cortical connections rather than to the elaboration of projection connections.

Developmental delay is a key feature of human evolution [22] and is thought to play a role in the emergence of human-specific cognitive abilities through an extended period of high neuronal plasticity [13, 23-24]. Delayed development of the human brain may affect its function by rendering it more susceptible to the influence of postnatal experiences [24]. Similarly, a recent imaging study of brain structure compared the maturity of cortical folds of human brains with that of macaque brains [25]. That study hypothesized that high-order association areas in postnatal development benefit from remaining less mature during the early human postnatal period. In this manner, the influence of postnatal experiences on the development of selected brain regions is increased. Therefore, our results suggest that less mature prefrontal connections during early infancy and a protracted developmental period of the prefrontal network in chimpanzee brains will likewise result in a strong influence of postnatal experiences on functional and behavioral outcomes.

A series of cognitive studies with the same three chimpanzee subjects employed in the current study revealed that chimpanzee infants share some common features with human infants with regard to cognitive development during early infancy (see [26] and references therein). These chimpanzee infants, similarly to human infants, demonstrated

closed dyadic relationships (dyadic infant-adult, or object interactions) with their mothers during early infancy, based on mutual gaze and social smiling [27]. Such interactions are not observed in macaque infants. Also similarly to humans, the three chimpanzees demonstrated first object-object manipulation, a precursor to tool use, during early infancy [28]. This was followed by first tool use, which consisted of the employment of a probing tool to obtain honey through a small hole [29]. Taken together, we hypothesize that brain connection development, particularly in the prefrontal portion, may have been under intense evolutionary pressure to remain immature, producing a brain that is more susceptible to acquiring chimpanzee-human-specific social and technical skills based on early postnatal experiences.

However, those studies [26-29] reported crucial differences between chimpanzees and humans in behavioral and cognitive development during early infancy and the juvenile stage. Triadic interactions (triadic infant-adult and object interactions), based on sharing attention and additional important social interactions with the Theory of Mind [30], develop in humans between 9 months and 3 years (near the middle of early infancy and the first half of late infancy, respectively) [31]. Triadic interactions were lacking in the three chimpanzees even at the later juvenile stage [26-27]. Furthermore, chimpanzees have a limited capacity to combine many objects in a hierarchical order, whereas humans can develop hierarchical combinatorial manipulations at infinite levels [28]. On the other hand, the working memory of chimpanzees aged 4 to 5 years (the first half of the juvenile stage) has more potential for expansion than that of chimpanzee and human adults [32]. One interpretation of this intriguing finding suggests that humans lose the memory skills over time that are required to obtain language-related skills, conceptual representation, chunking (breaking down information or details into smaller parts), hierarchical organization, and

syntactic rules [26, 32], because brain volume capacity was limited at a certain point in human life [33-38].

In this context, we hypothesize that the dramatic increase of the prefrontal WM volume during human infancy, which was not observed in chimpanzees, corresponded with opportunities for human-specific social learning and acquisition of technical and linguistic skills. Furthermore, the protracted period of the prefrontal connection elaboration in chimpanzees appeared to only affect specific social and cognitive processes based on direct and dyadic subject-object relationships and did not impart behaviors based on social reference frameworks.

In conclusion, our results suggest that, as groundwork for the reformatting of these expanded brain regions in humans, the prefrontal reciprocal connections to the posterior brain regions became enhanced during the course of evolution. This enhancement likely occurred through an extension of the period of prefrontal connection maturation in the common lineage after the last common ancestor shared by chimpanzees and modern humans split from macaques. However, the lineage leading to modern humans has undergone substantial evolutionary modifications, resulting in the rapid developmental of the prefrontal connections during infancy. This likely facilitates the development of complex social interactions and the shaping of neuronal connectivity through the acquisition of experience-dependent knowledge and skills.

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Figure Legends

Figure 1. An ontogenetic series of the whole cerebrum and the prefrontal portion in a chimpanzee brain during early infancy and the juvenile stage

MRI brain images were acquired from three young chimpanzees as they developed between the ages of 6 months and 6 years and also from two adult chimpanzees. MRI brain images were aligned by age with the images from a representative young chimpanzee (Pal) and an adult (Reo) for comparison. (A) T1-weighted anatomical brain images. (B) Segmentation of the cerebrum: white matter (WM), gray matter (GM), and cerebrospinal fluid (CSF). (C) Three-dimensional renderings of the cerebrum from left and right lateral views, including the cortical portion designated as the prefrontal portion. The color bar represents the developmental stages based on combined dental eruption and sexual maturation. The developmental stage in chimpanzees corresponds to early infancy (red), late infancy (orange), juvenile stage (green), and adult stage (purple). Also see **Supplemental Experimental Procedures** for the more detailed account of the experimental procedures (subjects, image acquisition, and image processing), and **Supplemental Discussion** for the demarcation of the prefrontal portion. **Supplemental Experimental Procedures** and **Figure S1** for the definitions of the developmental stages in chimpanzees.

Figure 2. Volumetric changes in the prefrontal and nonprefrontal portions during early infancy and the juvenile stage in chimpanzees

Age-related changes in the total volume (WM plus GM) (A), WM volume (B), and GM volume (C) in the prefrontal and non-prefrontal portions in chimpanzees (Ayumu, Cleo, and Pal), respectively. The color bar represents the developmental stages based on combined dental eruption and sexual maturation. The developmental stage in chimpanzees

corresponds to early infancy (red), late infancy (orange), and the juvenile stage (green). The black dotted lines represent the study range in chimpanzees. Also see **Supplemental Experimental Procedures** for the statistical analysis, **Supplemental Results**, **Figure S2**, and **Figure S3** for the comparison with the results of the total, WM and GM volumes of the prefrontal and non-prefrontal portions in humans and macaques, **Supplemental Discussion** for the demarcation of the prefrontal portion, and **Figure S1** for the definitions of the developmental stages in chimpanzees.

Figure 3. Evaluation of WM volumes relative to the adult volume in the prefrontal and non-prefrontal portions during early infancy and the juvenile stage

Age-related changes in the relative WM volumes are shown in the prefrontal and non-prefrontal portions in chimpanzees (Ayumu, Cleo, and Pal) (A), humans (n = 28) (B), and rhesus macaques (n = 37) (C), respectively. The color bar represents the developmental stages based on combined dental eruption and sexual maturation. The developmental stage in chimpanzees, humans, and macaques corresponds to early infancy (red), late infancy (orange), juvenile stage (green), puberty (dark green), and adult stage (purple). The black dotted lines represent the study ranges in chimpanzees, humans, and macaques. Also see **Supplemental Experimental Procedures** for the developmental trajectories in humans and macaques and the statistical analysis, **Supplemental Discussion** for the demarcation of the prefrontal portion and the demarcation of the cerebrum and the prefrontal portions in humans and macaques, and **Figure S1** for the definitions of the developmental stage in chimpanzees, humans, and macaques.

Figure 4. Evaluation of proportional WM volumes compared with the adult value in the prefrontal and non-prefrontal portions during early infancy and the juvenile stage

Age-related changes in the proportional WM volumes in the prefrontal and non-prefrontal portions in chimpanzees (Ayumu, Cleo, and Pal) (A), humans (n = 28) (B), and rhesus macaques (n = 37) (C), respectively. The color bar represents the developmental stages based on combined dental eruption and sexual maturation. The developmental stage in chimpanzees, humans, and macaques corresponds to early infancy (red), late infancy (orange), the juvenile stage (green), puberty (dark green), and adult stage (purple). The black dotted lines represent the study ranges in chimpanzees, humans, and macaques. Also see **Supplemental Experimental Procedures** for the developmental trajectories in humans and macaques and the statistical analysis, **Supplemental Results** for the more detailed results about the proportional WM volumes of the prefrontal and non-prefrontal portions in humans and macaques, **Supplemental Discussion** for the demarcation of the prefrontal portion and the demarcation of the cerebrum and the prefrontal portion in humans and macaques, and **Figure S1** for the definitions of the developmental stages in chimpanzees, humans, and macaques.

Figure 1

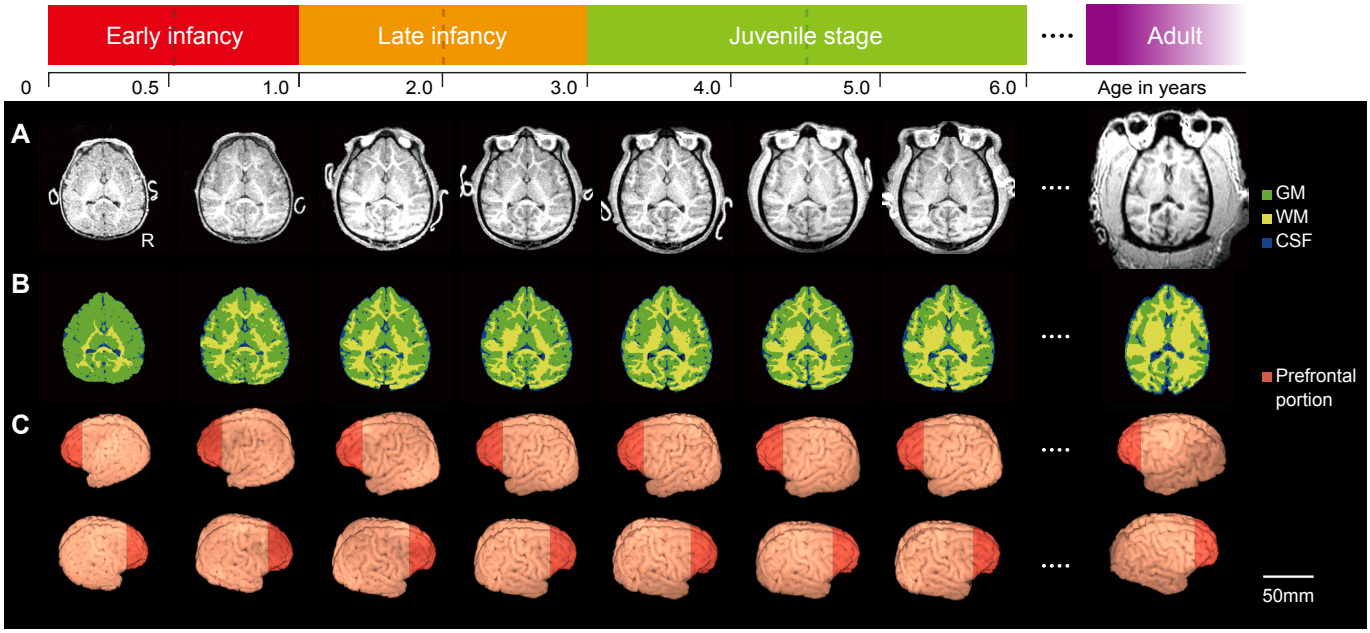


Figure 2

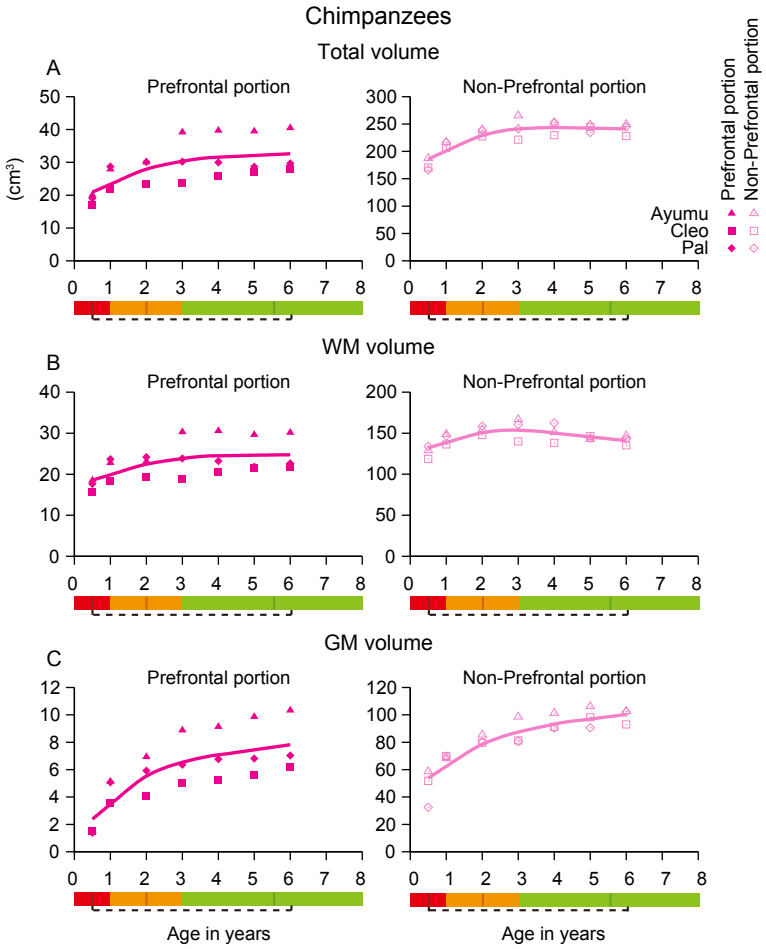


Figure 3

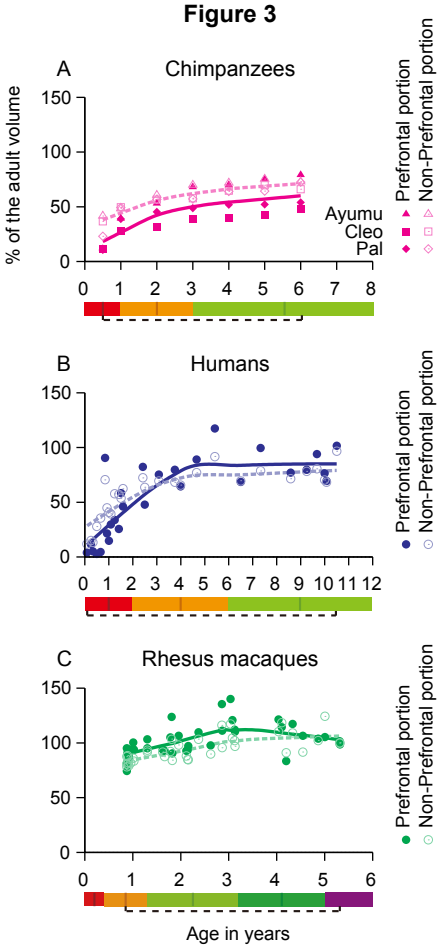
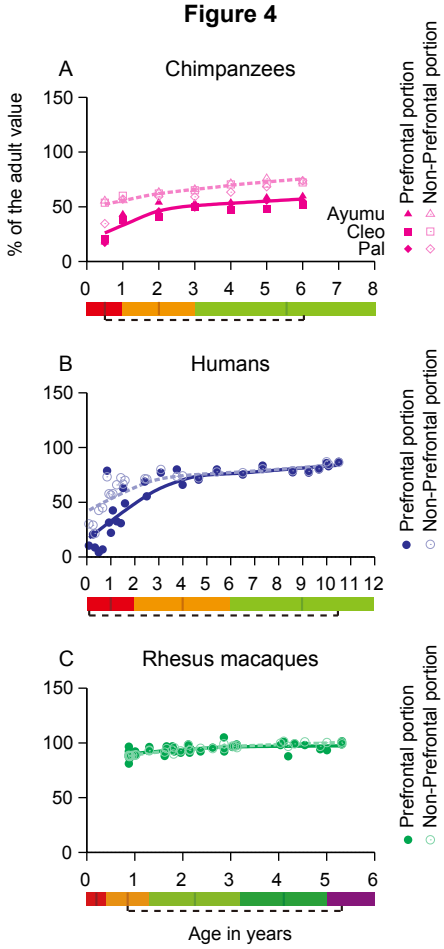


Figure 4



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Supplemental Information

Differential Prefrontal White Matter Development in Chimpanzees and Humans

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Supplemental Inventory

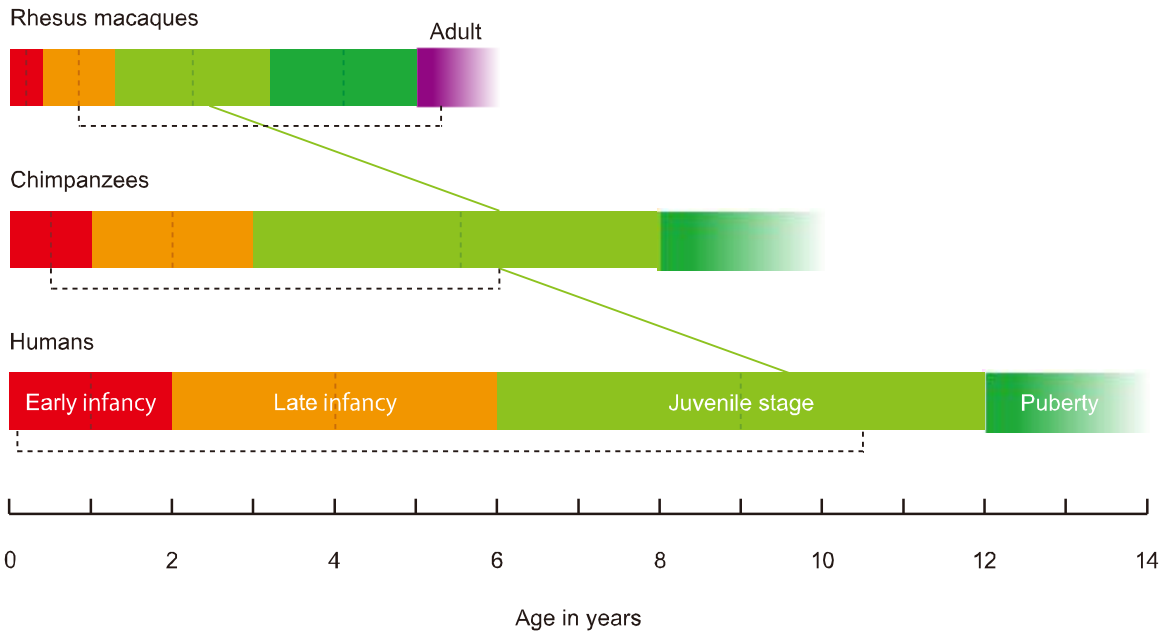

Figure S1. Developmental stages are based on combined dental eruption and sexual maturation in chimpanzees, humans, and rhesus macaques

The color bar represents the developmental stages, which correspond to early infancy (red), late infancy (orange), juvenile stage (green), puberty (dark green), and adult stage (purple). Here, we compared the developmental trajectories of brain volumes in the three species within the range indicated by the black dotted lines. The green solid lines represent the developmental stage in humans and macaques corresponding to 6 years of age (the second half of the juvenile stage) in chimpanzees. Also see Supplemental Experimental Procedures for a more detailed account of the definitions of the developmental stages in chimpanzees, humans, and macaques.

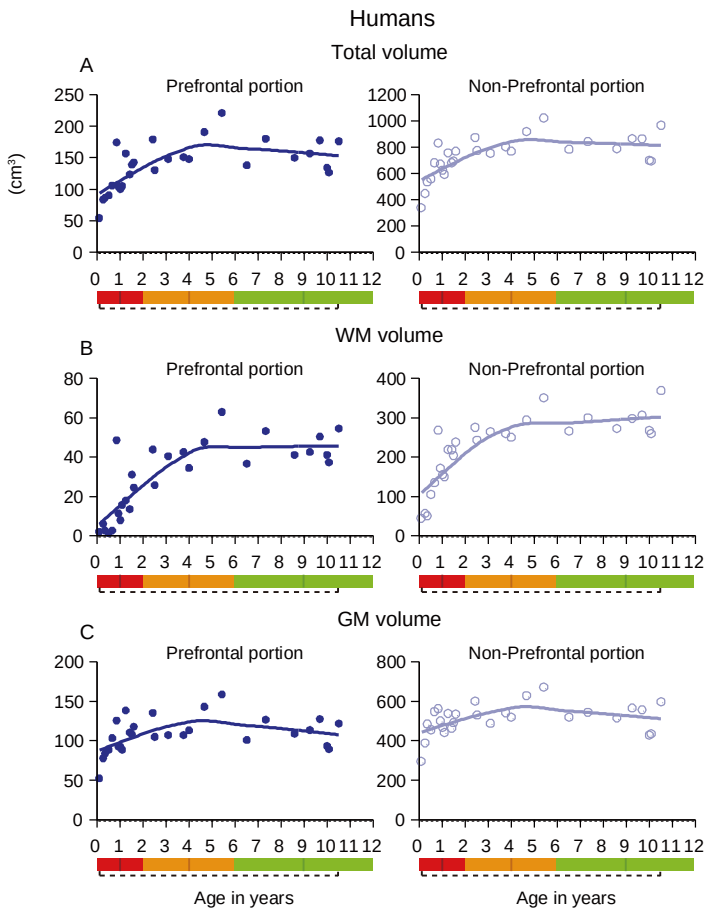

Figure S2. Volumetric changes in the prefrontal and nonprefrontal portions during early infancy and the juvenile stage in humans

Age-related changes in the total volume (WM plus GM) (A), WM volume (B), and GM volume (C) in the prefrontal and nonprefrontal portions in humans, respectively (n = 28). The color bars below the graphs represent developmental stages based on combined dental eruption and sexual maturation. The developmental stages in humans correspond to early infancy (red), late infancy (orange), and the juvenile stage (green). The black dashed lines represent the study range in humans. Also see Supplemental Experimental Procedures for the developmental trajectories in humans and the statistical analysis, Supplemental Discussion for the demarcation of the prefrontal portion and the demarcation of the cerebrum and the prefrontal portions in humans, and Figure S1 for the definitions of the developmental stages in humans.

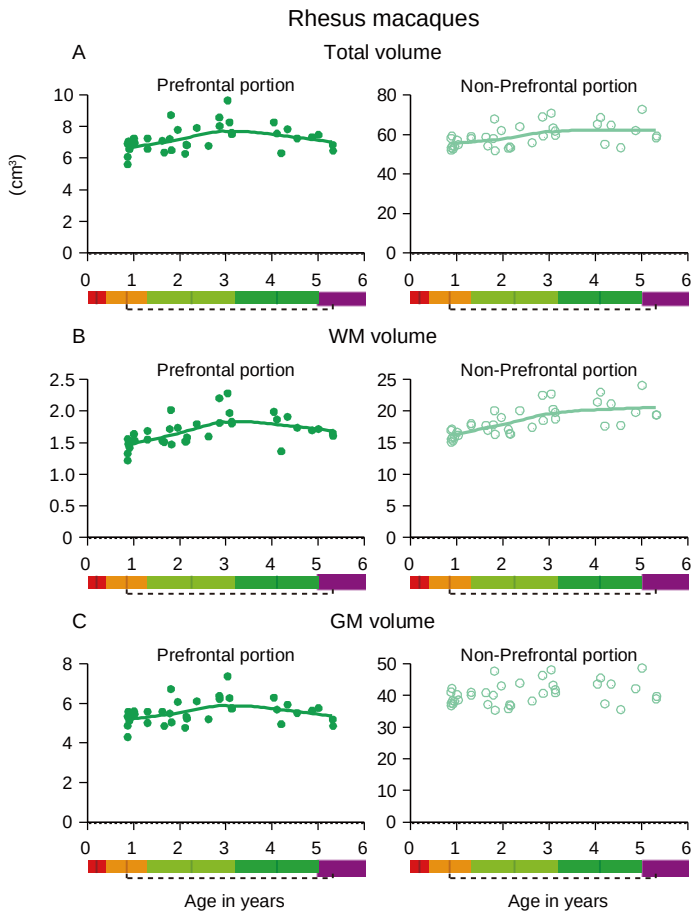

Figure S3. Volumetric changes in the prefrontal and nonprefrontal portions during late infancy and the adult stage in rhesus macaques

Age-related changes in the total volume (WM plus GM) (A), WM volume (B), and GM volume (C) in the prefrontal and nonprefrontal portions in rhesus macaques, respectively (n = 37). The color bars below the graphs represent the developmental stages based on combined dental eruption and sexual maturation. The developmental stages in macaques correspond to early infancy (red), late infancy (orange), juvenile stage (green), puberty (dark green), and adult stage (purple). The black dashed lines represent the study range in macaques. Also see Supplemental Experimental Procedures for the developmental trajectories in macaques and the statistical analysis, Supplemental Discussion for the demarcation of the prefrontal portion and the demarcation of the cerebrum and the prefrontal portions in macaques, and Figure S1 for the definitions of the developmental stages in macaques.

Table S1. Age and Total, Gray, and White Matter Volume

Subject (Sex)	Age (yr)	Nonprefrontal portion (cm^3) Total	Gray matter	White matter	Prefrontal portion (cm^3) Total	Gray matter	White matter
Ayumu (M)	0.5	187.4	128.9	58.5	20.0	18.5	1.5
	1	216.8	148.7	68.1	27.9	22.7	5.1
	2	239.6	154.3	85.3	30.0	23.1	6.9
	3	265.1	166.7	98.4	39.1	30.3	8.9
	4	252.0	150.9	101.2	39.7	30.5	9.1
	5	248.5	142.4	106.1	39.5	29.6	9.9
	6	249.4	146.8	102.6	40.4	30.1	10.3
Cleo (F)	0.5	170.5	118.8	51.7	17.2	15.6	1.5
	1	206.2	136.3	69.9	22.0	18.4	3.6
	2	227.4	147.7	79.7	23.3	19.3	4.1
	3	221.4	140.1	81.3	23.8	18.8	5.1
	4	229.6	138.3	91.2	25.7	20.5	5.2
	5	244.8	146.5	98.3	27.2	21.6	5.6
	6	228.1	135.1	93.0	28.1	21.8	6.2
Pal (F)	0.5	166.4	133.9	32.5	19.0	17.7	1.4
	1	216.1	146.5	69.6	28.7	23.7	5.1
	2	238.6	158.6	80.0	30.1	24.2	5.9
	3	241.2	160.7	80.5	30.2	23.8	6.4
	4	252.9	162.5	90.4	30.0	23.2	6.8
	5	234.6	144.0	90.6	28.7	21.9	6.8
	6	245.1	142.9	102.1	29.7	22.7	7.0
Adult chimpanzees							
Reo (M)	23	233.6	113.7	119.9	28.6	17.4	11.2
Ai (F)	32	279.0	117.0	162.0	33.3	18.7	14.6
Shoenemann et al. (2005)							
Merv (M)	-	334.7	135.4	199.4	44.4	23.4	21.0
Laz (M)	-	238.0	111.1	126.9	20.9	10.5	10.3
Jimmy Cater	-	263.8	130.4	133.3	28.5	19.1	9.5
Mary (F)	-	271.8	112.2	159.6	23.0	11.1	11.9
Lulu (F)	-	248.1	102.0	146.0	24.6	15.0	9.6
Kengree (F)	-	310.7	152.1	158.6	40.6	24.4	16.2
Average	-	280.2	126.2	154.0	30.5	17.4	13.0
±SD	-	34.7	16.5	25.0	8.4	5.1	4.0

Supplemental Experimental Procedures

Subjects

Three growing chimpanzees (*Pan troglodytes*), named Ayumu (male), Cleo (female), and Pal (female), and two adult chimpanzees, named Reo (male) and Ai (female), participated in this study. All subjects lived within a social group of 14 individuals in an enriched environment at the Primate Research Institute, Kyoto University (KUPRI) [26, 39]. Our three growing study chimpanzees were born on 24 April, 2000, 19 June, 2000, and 9 August, 2000, respectively. They were raised by their biological mothers. All protocols were approved by the Committee for the Care and Use of Laboratory Primates of KUPRI.

Image Acquisition

We acquired three-dimensional T1-weighted whole brain images from the three growing chimpanzees between the ages of 6 months and 6 years with a 0.2 Tesla MR imager (Signa Profile; General Electric) using the same three-dimensional spoiled-gradient recalled echo (3D SPGR) imaging sequence. For comparison, adult data were obtained from two chimpanzees. Prior to scanning, the three growing and two adult chimpanzees were anesthetized with ketamine (3.5 mg/kg) and medetomidine (0.035 mg/kg) and then transported to the MRI scanner. The subjects remained anesthetized for the duration of the scans and during transportation between their home cage and the scanner (total time anesthetized ≈2 h). They were placed in the scanner chamber in a supine position with their heads fitted inside either the extremity (for the growing chimpanzees) or the head coil (for the adult chimpanzees). The 3D SPGR acquisition sequence was obtained with the following acquisition parameters: repetition time (TR): 46 ms; echo time (TE): 10 ms; flip angle: 60°; slice thickness: 1.0 mm; field of view: 14–16 cm (for the growing chimpanzees) or 24 cm (for the adult chimpanzees); matrix size, 256 × 256; number of excitations: two.

Image Processing

The MR images for each individual were analyzed using a series of manual and automated procedures as follows: (i) All images were analyzed using Analyze 9.0 software (Mayo Clinic, Mayo Foundation, Rochester, MN, USA) and converted to cubic voxel dimensions of 0.55 mm using a cubic spline interpolation algorithm. (ii) Brain image volumes were realigned to a standard anatomical orientation with the transaxial plane parallel to the anterior commissure-posterior commissure line and perpendicular to the interhemispheric fissure. (iii) The cerebral portion of the brain was semi-manually extracted using BET [40] in the FSL package (version 4.1; www.fmrib.ox.ac.uk/fsl) [8]. Nonbrain tissues (scalp, orbits) were removed, followed by cerebellar and brain stem tissues (midbrain, pons, medulla), which were obtained as follows. Noncerebral tissues were removed in the coronal plane, starting at the most posterior point and proceeding anteriorly until no obvious break was evident between the midbrain and thalamus [6]. Next, noncerebral tissues were removed in the axial plane according to a previous study [6], starting at the most inferior slice and proceeding superiorly until no obvious break was evident between the midbrain and the posterior limb of the internal capsule (the transition between the cerebral peduncle and the posterior limb of the internal capsule). Thus, the cerebral portion included most of the deep central gray matter (GM) (caudate nuclei, putamen, globus pallidus, lentiform nuclei, thalamus, and intervening white matter (WM)), the hippocampus and amygdala in all subjects. (iv) MRI data were spatially smoothed using SUSAN [41] in FSL, which reduces noise, ideally without blurring the underlying images. (v) Each brain volume was segmented into GM, WM, and cerebrospinal fluid (CSF) based on signal intensity, while also correcting for magnetic field in homogeneities using FAST [42] in FSL (Figure 1). This method was based on a hidden Markov random field model and an associated expectation-maximization algorithm. The tissue segmentation samples at each developmental stage, particularly in infancy, were reviewed by a neuroradiologist (H.T.) to ascertain if the GM/WM border determined by FAST was accurate. Next, all results of the GM and WM segmentation were reviewed and corrected semi-automatically, as necessary. (vi) The prefrontal portion was defined as consisting of all coronal slices anterior to the corpus callosum of the cerebrum, as a proxy for the prefrontal volume on MRI data, in accordance with previous studies [4-7]. The nonprefrontal portion corresponded to the remainder of the cerebrum. The rationale for the definition of the prefrontal portion is explained in Supplemental Discussion. (vii) The absolute volumes of GM and WM in the prefrontal and nonprefrontal portions of the cerebrum were measured, and the volumes of the regions of interest were calculated from an automatic count of the number of voxels per mm^3 using

FSLUTILS in FSL (Table S1). The total volume of each portion corresponded to the sum of the GM and WM volume of that portion.

Two image analysts (T.S. and H.M.), blind to the gender and age of the subjects, semi-manually traced and measured the entire cerebrum. T.S. identified the landmarks of the cerebrum in all brain images in consultation with a neuroradiologist (H.T.) and an anatomical expert (A.M. and M.M.). An interrater reliability analysis was conducted to compare the cerebral measurements obtained by T.S. with a sample of brain scans measured by H.M. Ten brain scans were randomly selected for analysis. The Pearson's correlation coefficient for the comparison of the results obtained by T.S. and H.M. was $r = 0.91$, $P < 0.01$.

Comparison with the Developmental Trajectories in Humans and Macaques

Direct comparison with developmental trajectories in humans (*Homo sapiens*) and rhesus macaques (*Macaca mulatta*) allowed us to identify human-chimpanzee-macaque-shared features, human-chimpanzee-shared features, and human-specific features. Through comparison with human data [9] (Matsui et al., unpublished data; these data were presented as an abstract at the 20th annual Rotman Research Institute Conference- "Frontal Lobes" in 2010) and macaque data [3], we sought to assess whether humans have specialized developmental patterns of the brain volume relative to chimpanzees and macaques, and if so, when the differences in the developmental patterns emerge.

In the human cross-sectional data, we analyzed the age-related brain volume data from 28 healthy Japanese children (14 males, 14 females) during the period between 1 month and 10.5 years of age (see details in [9] and in the abstract of Matsui et al., 2010). The comparison to human adult volumes is based on the data from 16 healthy adults who served as controls in the abstract of Matsui et al. (2010). Adult subject characteristics were as follows: mean (SD) age, 21.3 (1.8) years; female/male ratio, 50% male. In the macaque cross-sectional data, we analyzed the age-related brain volume data from 37 normal macaques (20 males, 17 females) during the period between 10 months and 5.3 years (see details in [3]). All macaque subjects were generated from a large monkey-breeding colony and were raised by their biological mothers. In the statistical analyses in this study, we performed the same procedures to obtain data from macaques, humans, and chimpanzees.

Definitions of the Developmental Stages in Chimpanzees, Humans, and Macaques

In this study, we chose developmental indicators based on combined dental eruption and sexual maturation for inter-specific comparisons. In the developmental stages based on dental eruption, we defined three developmental stages: "early infancy", "late infancy", and the "juvenile stage" (Figure S1) [43-45]. These stages were demarcated by the eruption of the first deciduous tooth and the eruption of the first permanent tooth. The juvenile stage ends at sexual maturation (menarche, first ejaculation) [46-49]. In chimpanzees, these developmental stages were ~1 year of age, ~3 years of age, ~8 years of age; in macaques, ~0.4 years of age, ~1.3 years of age, ~3.2 years of age; in humans, ~2 years of age, ~6 years of age, ~12 years of age.

Statistical Analysis

Total and Prefrontal Tissue Volumes

All statistical analyses were performed using SPSS 19 (SPSS, Chicago) and R 2.11.1 (http://www.r-project.org/) software. Hypothesis tests for model building were based on *F* statistics. At first, linear, quadratic, or cubic polynomial regression models were fitted by age using SPSS 19 to identify the brain developmental pattern of volume in the prefrontal and nonprefrontal portions. If a cubic model did not yield significant results, a quadratic model was tested; if a quadratic model did not yield significant results, a linear model was tested. Thus, a growth model was polynomial/nonlinear if either the cubic or the quadratic term significantly contributed to the regression equation. We used the likelihood function Akaike information criterion (AIC) to ensure effective model selection.

Secondly, using R 2.11.1 software, we fitted the data that showed nonlinear trajectories by locally weighted polynomial regression (LOESS) [50]. In this way, we were able to delineate age-related volume changes by applying the curve fitting suggested in previous human studies [51-52] without enforcing a common parametric function on the full data set, as is the case with linear polynomial models. For the fit at age X, the fit is made using values in a neighbourhood that includes a proportion alpha, and for alpha <

1, the neighborhood includes a proportion alpha of the values. Data were fitted in 4 interactions with alpha = 0.75. Observed and fitted values of the total, WM, and GM volumes in the prefrontal and nonprefrontal portions were plotted as a function of age to display age-related variability. All statistical hypothesis tests were conducted at a significance level of 0.05.

To assess the difference of WM volume development between prefrontal and nonprefrontal portions, we calculated the relative WM volume as a percentage of the adult WM volume in each portion. To adequately describe the variability in the data among adult chimpanzees as compared to that among young chimpanzees, we added data about the GM and WM volumes of the prefrontal and nonprefrontal portions of six adult chimpanzees from a previous study [6] to the present data about two adult chimpanzees. All statistical hypothesis tests were conducted at a significance level of 0.05.

Proportional Growth of WM Volume

The combined developmental pattern of the prefrontal and nonprefrontal portions in chimpanzees differed from the pattern in both humans and macaques. Chimpanzees, like humans, showed a relatively protracted development of the prefrontal total volume compared with macaques during infancy and the juvenile stage. The prefrontal total volume in chimpanzees and humans continued to increase near the beginning of puberty (Figure 2A and Figure S2A), whereas the macaque prefrontal volume more quickly reached a developmental peak at 2.9 years (near the end of the juvenile stage) and then decreased from puberty onward (Figure S3A). In chimpanzees, the developmental trajectories of the total volume in the prefrontal portion differed from those of the nonprefrontal portion. Human developmental trajectories, by contrast, showed increases in the total volume of both prefrontal and nonprefrontal portions (Figure S2A). The total nonprefrontal volume of the chimpanzee cerebrum increased at a relatively slow rate until around 4 years (the first half of the juvenile stage) and declined thereafter, whereas the total prefrontal volume continued to gradually increase with age across the early infancy and the juvenile stages (Figure 2A).

Differences in the prefrontal and nonprefrontal developmental patterns of WM volume appear to greatly influence the differences in the total volume of the adult prefrontal portion. Therefore, to elucidate species-specific variations in chimpanzees, humans, and macaques, we evaluated the proportional growth of the WM volume relative to the total volume of the developing cerebrum and compared the result to the adult value. We calculated the proportional WM volume relative to the total volume and compared the result with the adult value by dividing the WM volume expressed as a percentage of the total volume of GM plus WM in the prefrontal and nonprefrontal portions by the adult percentage. The proportional WM volume of the prefrontal and nonprefrontal portions increased significantly between 6 months and 6 years, following a cubic and quadratic curve, respectively. Analysis of covariance (ANCOVA) was used to compare the developmental trajectory of the proportional WM volumes between the prefrontal and nonprefrontal portions over this age period. All statistical hypothesis tests were conducted at a significance level of 0.05.

Supplemental Results

Total Volumes of the Prefrontal and Nonprefrontal Portions of the Human Cerebrum

The total volumes of each of the prefrontal and nonprefrontal portions increased nonlinearly from near the onset of early infancy to the second half of the juvenile stage (1 month to 10.5 years) ($F = 12.76$; cubic effect, $P < 0.0001$; $F = 16.12$; cubic effect, $P < 0.0001$) (Figure S2A). The WM volumes in the prefrontal and nonprefrontal portions increased nonlinearly during this developmental stage, respectively ($F = 22.89$; cubic effect, $P < 0.0001$; $F = 38.89$; cubic effect, $P < 0.0001$) (Figure S2B). The GM volumes in each portion also increased nonlinearly during this period ($F = 6.31$; cubic effect, $P < 0.005$; $F = 5.39$; cubic effect, $P < 0.01$) (Figure S2C).

Proportional Growth of WM Volume in Humans

The proportional WM volume of the prefrontal and nonprefrontal portions increased nonlinearly from near the onset of early infancy to the second half of the juvenile stage (1 month to 10.5 years), respectively ($F = 33.18$; cubic effect, $P < 0.0001$; $F = 41.41$; cubic effect, $P < 0.0001$ (Figure 4B). The proportional WM volume in the prefrontal portion was significantly smaller than that in the nonprefrontal portion (repeated measures ANCOVA; $F = 82.55$, $P < 0.0001$).

Total Volumes of the Prefrontal and Nonprefrontal Portions of the Macaque Cerebrum

The total volumes of each of the prefrontal and nonprefrontal portions increased nonlinearly from the middle of late infancy to near the beginning of adult stage (10 months to 5.3 years) ($F = 8.38$; quadratic effect, $P < 0.005$; $F = 6.29$; quadratic effect, $P < 0.005$) (Figure S3A). The WM volumes in the prefrontal and nonprefrontal portions also increased nonlinearly during this developmental stage, respectively ($F = 13.11$; quadratic effect, $P < 0.0001$; $F = 19.92$; quadratic effect, $P < 0.0001$ (Figure S3B). There were no significant age-related changes in the nonprefrontal GM volume, whereas the prefrontal GM volume increased nonlinearly during this period ($F = 2.46$; quadratic effect, $P = 0.1008$; $F = 6.65$; quadratic effect, $P < 0.005$) (Figure S3C).

Proportional Growth of the WM Volume in Macaques

The proportional WM volume of the prefrontal and nonprefrontal portions in macaques increased nonlinearly from the middle of late infancy and near the beginning of adult stage (10 months to 5.3 years), respectively ($F = 9.16$; quadratic effect, $P < 0.001$; $F = 109.02$; quadratic effect, $P < 0.0001$) (Figure 4C). There was no significant difference between the middle of late infancy compared with the end of the juvenile stage (10 months to 3.2 years) (ANCOVA, $F = 0.84$; $P = 0.3650$).

Supplemental Discussion

Demarcation of the Prefrontal Portion

We defined the prefrontal portion of the cerebrum as all coronal slices anterior to the corpus callosum, in accordance with previous studies [4-7]. This anatomical definition of the prefrontal region remains controversial and may in fact underestimate the extent of the prefrontal matter in chimpanzees and other primates, based on cytoarchitectural maps [10, 53-54] and discussed by Sherwood et al. [18]. However, this proxy measure of the prefrontal region is used in MRI studies of pediatric brain development given that formation of the cerebral sulci, fissures, and gyri, all gross anatomical landmarks of brain development, have not yet been characterized in the early postnatal period in humans [4, 5, 7]. Thus, this segmentation scheme was applied to compare the developmental patterns of the prefrontal volume during infancy and the juvenile stage in chimpanzees. Nonetheless, the possibility remains that alternative delineation criteria of the prefrontal region may have lead to different results.

Demarcation of the Cerebrum and the Prefrontal Portion in Humans and Macaques

Although the demarcations of the entire cerebrum and the prefrontal portion in human brains were similar to those in chimpanzee brains, those of macaque brains were distinctly different from those of chimpanzees and humans. Unlike in chimpanzee and human studies, a portion of the midbrain (namely the superior and inferior collicui) was included in the cerebrum in the macaque study because the boundary between the subcortical exclusion area and the medulla and the pons was immediately superior to the middle cerebellar peduncle [3]. Moreover, the estimation of the prefrontal region in the macaque study differed somewhat from the prefrontal estimation in the chimpanzee and human studies. The posterior border of the prefrontal region in macaques was defined as the arcuate sulcus, as opposed to the anterior extent of the corpus callosum, termed the genu, in chimpanzees and humans [3]. Moreover, data collected from humans and macaques were obtained from cross-sectional imaging studies [3, 9] (abstract of Matsui et al., 2010), unlike the data collected from chimpanzees, which was obtained from a longitudinal imaging study. However, these discrepancies are unlikely to appreciably influence the comparison of developmental trajectories of brain tissues among chimpanzees, humans, and macaques, because the volumetric differences that resulted in these discrepancies appear to be subtle. In fact, previous imaging studies that directly compared the developmental patterns of humans and nonhuman primates indicated that each of these species had characteristic features despite the fact that there were differences in the type of investigation (cross-sectional or longitudinal investigation), the anatomical demarcations of the brain, and the statistical analysis [3, 55-56]. It is important to ensure that these discrepancies do not lead to contradictory results in the future. Nonetheless, at the present time, our study is the first and most important study to directly compare the developmental trajectories of humans and nonhuman primates using the same statistical analysis throughout.

More Protracted WM Development in Chimpanzees Than in Humans

Interestingly, we also found that the WM volumes of the adult volume of the chimpanzee cerebrum, especially the prefrontal portion in the second half of the juvenile stage (6 years), were much smaller than those of the human cerebrum at almost the same developmental stage (10.5 years) (Figure 3A-B). This could be caused by differences in the rates of WM volume increases during infancy between the two species. The WM volume of humans, especially in the prefrontal portion, increased more dramatically than that of chimpanzees (Figure 2B and Figure S2B). This means that the reciprocal connection between cortical areas, especially the prefrontal portion to the posterior cortical areas, is more remarkably enhanced in human infancy than in chimpanzee infancy. According to several previous human brain imaging studies [57-58], the increase in human prefrontal WM volume continues beyond adolescence into early adulthood. However, at present, it is not clear whether the development of WM volumes is really more protracted in chimpanzees than in humans because we only have data for chimpanzee brain development during pre-puberty. A definite conclusion regarding this issue must wait for the results of an ongoing longitudinal study.

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